PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. 301-436-2151/2147 FAX 301-436-2672

SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY: Maine DMR Boothbay Harbor PSP Laboratory		
ADDRESS: 194 McKown Point Road, P.O. Box 8, West Boothbay Harbor, ME. 04575-0008		
TELEPHONE:	**FAX:**	**EMAIL:**
DATE OF EVALUATION: May 7, 2008	**DATE OF REPORT:**	**LAST EVALUATION:**

LABORATORY REPRESENTED BY: Darcie Couture	**TITLE:** Director, Biotoxin Monitoring
LABORATORY EVALUATION OFFICER: Linda Chandler	**SHELLFISH SPECIALIST:** **REGION:**
OTHER OFFICIALS PRESENT:	**TITLE:**
Laurie L Bean	MR Sci I Biotoxin

Items which do not conform are noted by:

C- Critical K – Key O – Other NA – Not Applicable Conformity is noted by a "√"

PART I – QUALITY ASSURANCE		
Code		**Item Description**
		Quality Assurance (QA) Plan
K	✓	1. Written Plan adequately covers all the following: (check √ those that apply) a. ✓ Organization of the laboratory. b. ✓ Staff training requirements. (needs to be expanded) c. ✓ Standard operating procedures. d. ✓ Internal quality control measures for equipment, calibration, maintenance, repair and performance. e. ✓ Laboratory safety. f. ✓ Quality assessment. g. ✓ Proper animal care.
C	✓	**2. QA plan implemented.**
		1.2 Work Area
O	✓	1. Adequate for workload and storage.
O	✓	2. Clean and well lighted.
O	✓	3. Adequate temperature control.
O	✓	4. All work surfaces are nonporous and easily cleaned.
C	✓	**5. A separate, quiet area with adequate temperature control for mice acclimation and injection is maintained.**
		1.3 Laboratory Equipment
O	NA	1. The pH meter has a standard accuracy of 0.1 pH unit.
K	✓	2. pH paper in the appropriate range (i.e: 1-4) is used with minimum accuracy of 0.5 pH units.
K	NA	3. pH electrodes consist of pH half cell and reference half cell or equivalent combination electrode (free from Ag/AgCl or contains an ion exchange barrier to prevent passage of Ag ions into the medium which may result in inaccurate pH readings).
K	NA	4. pH meter is calibrated daily or with each use. Records maintained.
K	NA	5. Effect of temperature has been compensated for by an ATC probe or by manual adjustment.
K	NA	6. A minimum of two standard buffer solutions (2 & 7) are used to calibrate the pH meter. Standard buffer solutions are used once and discarded.
K	NA	7. Electrode efficiency is determined daily or with each use following either slope or millivolt procedure.
K		8. The balance provides a sensitivity of at least 0.1g at a load of 150 grams.
K		9. The balance calibration is checked monthly using NIST Class S or ASTM Class 1or 2 weights or equivalent. Records maintained.
K	✓	10. Refrigerator temperature is maintained between 0 and 4°C.
K	✓	11. Refrigerator temperature is monitored at least once daily. Records maintained.

Code		Item Description
K	✓	12. Freezer temperature is maintained at –20°C or below.
O	✓	13. Freezer temperature is monitored at least once daily. Record maintained.
O	✓	14. All glassware is clean.
O	✓	15. Once during each day of washing, several pieces of glassware from each batch washed are tested for residual detergent with aqueous 0.04% bromthymol blue solution. Records are maintained.
		1.4 Reagent and Reference Solution Preparation and Storage
C	✓	**1. Opened PSP reference standard solution (100 µg/ml) is not stored.**
K	✓	2. PSP working standard solution (1 µg/ml) and all dilutions are prepared with dilute HCl, pH 3 water, using 'Class A' volumetric glassware (flasks and pipets) or prepared gravimetrically.
K	✓	3. Refrigerated storage of PSP working standard solution (1 µg/ml) does not exceed 6 months and is checked gravimetrically for evaporation loss.
K	✓	4. PSP working dilutions are discarded after use.
K	✓	5. Make up water is distilled or deionized (*circle one*) and exceeds 0.5 megohm resistance or is less than 2 µSiemens/cm conductivity at 25°C to be tested and recorded monthly for resistance or conductivity *(circle the appropriate)*.
O	✓	6. Make up water is analyzed for residual chlorine monthly and is at a nondetectable level ($\leq$ 0.1 ppm). Records maintained.
K	✓	7. Make up water is free from trace (< 0.5 mg/l) dissolved metals specifically Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content $\leq$1.0 mg/l. Records maintained.
O	✓	8. Makeup water contains < 1000 CFU/ml as determined monthly using the heterotrophic plate count method. Records maintained
		1.5 Collection and Transportation of Samples
O	✓	1. Shellstock are collected in clean, waterproof, puncture resistant containers.
K	✓	2. Samples are appropriately labeled with the collector's name, harvest area and time and date of collection.
K	✓	3. Immediately after collection, shellstock samples are placed in dry storage (cooler or equivalent) which is maintained between 0 and 10°C. for transport to the laboratory Upon receipt at the laboratory, samples are placed under refrigeration.

NSSP Form LAB-100 rev. 2001-02-14

Code		Item Description
K		4. The time from collection to completion of the bioassay should not exceed 24 hours. However, if there are significant transportation delays, then shellstock samples are processed immediately as follows (*circle the appropriate choice*): a. Washed, shucked, drained, frozen until extracted; b. Washed, shucked, drained, homogenized and frozen; c. Washed, shucked, drained, extracted, the supernatant decanted and refrigerated (**best choice**); or d. The laboratory has an appropriate contingency plan in place to handle samples which can't be analyzed within 24 hours due to transportation issues.
K		5. Frozen shucked product or homogenates are allowed to thaw completely and all liquid is included as part of the sample before being processed further.
PART II – EXAMINATION OF SHELLFISH FOR PSP TOXIN		
		2.1 Preparation of Sample
C	✓	1. At least 12 animals are used per sample or the laboratory has an appropriate contingency plan for dealing with non-typical species of shellfish.
O	✓	2. The outside of the shell is thoroughly cleaned with fresh water.
O	✓	3. Shellstock are opened by cutting adductor muscles.
O	✓	4. The inside of the shell is rinsed with fresh water to remove sand or other foreign material.
O	✓	5. Shellfish meats are removed from the shell by separating adductor muscles and tissue connecting at the hinge.
K	✓	6. Damage to the body of the mollusk is minimized in the process of opening.
O	✓	7. Shucked shellfish are drained on a #10 mesh sieve (or equivalent) without layering for 5 minutes.
K	✓	**8. Pieces of shell and drainage are discarded.**
C	NA	**9. Drained meats or thawed homogenates are blended at high speed until homogenous** (60 – 120 seconds).
		2.2 Extraction
K	✓	1. 100 grams of homogenized sample is weighed into a beaker.
K	✓	2. An equal amount of 0.1 N/0.18 N HCl is added to the homogenate and thoroughly mixed (*circle the appropriate normality*).
C	✓	**3. pH is checked and, if necessary adjusted to between pH 2.0 and 4.0.**
C	✓	**4. Adjustment of pH is made by the dropwise addition of either the acid (5 N HCl) or base (0.1N NaOH) while constantly stirring the mixture.**
C	✓	**5. The homogenate/acid mixture is promptly brought to a boil, 100 ± 1°C, then gently boiled for 5 minutes.**
O	✓	6. The homogenate/acid mixture is boiled under adequate ventilation (i.e. fume hood).
O	✓	7. The extract is cooled to room temperature. ice bath + room temperature
C	✓	**8. The pH of the extract is determined and adjusted, if necessary to between pH** 2 and 4, **preferably to pH 3 with the stirred dropwise addition of 5 N HCl to lower the pH or 0.1N NaOH to raise the pH.** usually have to adjust down

.11 – Laboratory Evaluation Checklist – **PSP** - 5

Code		Item Description
K	✓	9. The extract volume (or mass) is adjusted to 200 mls (or grams) with dilute HCl, pH 3 water.
K	✓	10. The extract is returned to the beaker, stirred to homogeneity and allowed to settle to remove particulates; or, if necessary, an aliquot of the stirred supernatant is centrifuged at 3,000 RPM for 5 minutes before injection.
K	✓	11. If mice cannot be injected immediately then the supernatant should be removed from the centrifuge tubes and refrigerated for up to 24 hours.
K	✓	12. Refrigerated extracts are allowed to reach ambient temperature before being bioassayed.
		2.3 Bioassay
O	✓	1. A 26-gauge hypodermic needle is used for injection.
K	✓	2. Healthy mice in the weight range of 17 –23 grams (19 – 21 grams preferable) from a stock colony are used for routine assays. Mice are not reused for bioassay. Stock strain used Swiss Webster CD-1 female Source of mice Charles River
C	✓	**3. Mice are allowed to acclimate for at least 24 hours prior to injection. In some cases up to 48 hours may be required.**
C	✓	**4. A conversion factor (CF) has been determined as** 0.21 **. Month and year when current CF determined ________________.**
C	✓	**5. CF value is checked weekly if assays are done on several days during the week, or, once each day that assays are performed if they are performed less than once per week.** **Date of most recent CF check ______________** **CF verified/CF not verified** *(Circle appropriate choice)* CF checks for 9/1 and 9/10 (missing) no CF check done when DT fell below 5 min or above 7 minutes; 4/12 and 5/15
C	NA	**6. If the CF is not verified, 5 additional mice are injected with the dilution used in the CF check to complete a group of 10 mice. Ten additional mice are also injected with this dilution to produce a second group of 10 mice. The CF is calculated for each group of 10 mice and averaged to give the CF to be used in sample toxicity calculations for the day's or week's work only. All subsequent work must make use of the original laboratory CF value unless this value continues to fail to be verified by routine CF checks.**
C	NA	**7. If the CF fails to be verified, the cause is investigated and the situation corrected. If the cause cannot be determined with reasonable certainty and fails > 3 times per year, the bioassay is restandardized.**
O	✓	8. Mice are weighed to the nearest 0.5 gram.
C	✓	**9. Mice are injected intrapertioneally with 1 ml of the acid extract.**
K	✓	10. For the CF check, at least 5 mice are used.
C	✓	**11. At least 3 mice are used per sample in routine assays.**
C	✓	**12. Elapsed time is accurately determined and recorded.**
K	✓	13. If death occurs, the time of death to the nearest second is noted by the last gasping breath.

NSSP Form LAB-100 rev. 2001-02-14

.11 – Laboratory Evaluation Checklist – **PSP** - 6

Code		Item Description
O	✓	14. Mice are carefully observed for up to 20 minutes after injection with periodic checks for a total of 60 minutes.
C	✓	**15. If median death time(2 out of 3 mice injected die) is < 5 minutes, a dilution is made with dilute HCl, pH 3 water, to obtain a median death time in the range of 5 to 7 minutes.**
		2.4 Calculation of Toxicity
C	✓	**1. The death time of each mouse is converted to mouse units (MU) using Sommer's Table (Table 6 *Recommended Procedures*, 4th edition). The death time of mice surviving beyond 60 minutes is considered to be < 0.875 MU.**
K	✓	2. A weight correction in MU is made for each mouse injected using Table 7 in *Recommended Procedures*, 4th edition.
C	✓	**3. The death time of each mouse in MU is multiplied by a weight correction in MU to give the corrected mouse unit (CMU) for each mouse.**
C	✓	**4. The median value of the array of corrected mouse units (CMU) is determined to give the median corrected mouse unit (MCMU).**
C	✓	**5. The concentration of toxin is determined by the formula, MCMU x CF X Dilution Factor X 200.**
C	✓	**6. Any value greater than 80 µg/100 grams of meat is actionable.**

NSSP Form LAB-100 rev. 2001-02-14

REFERENCES

1. Adams and Furfari, Evaluation of laboratory performance of the AOAC method for PSP toxin in shellfish, 1984. *J. Assoc. Off. Anal. Chem.* Vol 67, 6:1147-1148.
2. American Public Health Association. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition, 1970.
3. American Public Health Association. *Standard Method for the Examination of Dairy Products*, 16th Edition, 1992.
4. AOAC International. *Methods of Analysis*, 15th Edition, 1990.
5. APHA/WEF/AWWA. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, 1992.
6. Good laboratory practice.
7. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 1996. National Academy Press.
8. Personal communication with USFDA Washington Seafood Laboratory Branch, Office of Seafood, CFSAN, 1998-1999.

LABORATORY: PSP Boothbay	**DATE OF EVALUATION:** 5/8/08

SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY OF NONCONFORMITIES

Page	Item	Observation	Documentation Required
		No nonconformances noted. See recommendations for tweaks in operations in the evaluation report.	

LABORATORY STATUS

LABORATORY	**DATE**
Boothbay PSP	5/8/08

LABORATORY REPRESENTATIVE:

PARALYTIC SHELLFISH POISON COMPONENT: PARTS I and II

A. Results

Total # of Critical (C) Nonconformities _____

Total # of Key (K) Nonconformities _____

Total # of Critical, Key and Other (O) nonconformities 0

B. Criteria for Determining Laboratory Status of the PSP Component

1. **Does Not Conform Status** The PSP component of this laboratory is not in conformity with NSSP requirements if:
   A. The total # of Critical nonconformities is $\geq$ **3 or**
   B. The total # of Key nonconformities is $\geq$ **6 or**
   C. The total # of Critical, Key and Other is $\geq$ **10**

2. **Provisionally Conforms Status**: The PSP component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is $\geq$ 1 but < 3

C. Laboratory Status (*circle appropriate*)

Does Not Conform - Provisionally Conforms - (Conforms)

Acknowledgment by Laboratory Director/Supervisor:

All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____

Laboratory Signature: [signature] Date: 5-8-08

LEO Signature: [signature] Date: 5/8/08

NSSP Form LAB-100 rev. 2001-02-14